Figure 1

SEQ ID NO:1

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 121 gggttcttgg agaagaacct ggtcccagag gagcttgact gaccataaaa atgagtactg
 181 cagatgcact tgatgatgaa aacacattta aaatattagt tgcaacagat attcatcttg
 241 gatttatgga gaaagatgca gtcagaggaa atgatacgtt tgtaacactc gatgaaattt
 301 taagacttgc ccaggaaaat gaagtggatt ttattttgtt aggtggtgat ctttttcatg
 361 aaaataagcc ctcaaggaaa acattacata cctgcctcga gttattaaga aaatattgta
 421 tgggtgatcg gcctgtccag tttgaaattc tcagtgatca gtcagtcaac tttggtttta
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1981 gtagcaggaa ctcaaagact gctgtgtcag catctagaaa tatgtctatt atagatgcct
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2521 aataaagcta ccatttt
```

Figure 2

SEQ ID NO:2

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 181 lgsipderly rmfvnkkvtm lrpkedensw fnlfvihqnr skhgstnfip eqflddfidl
 241 viwgheheck iaptkneqql fyisqpgssv vtslspgeav kkhvgllrik grkmnmhkip
 301 lhtvrqffme divlanhpdi fnpdnpkvtq aiqsfcleki eemlenaere rlgnshqpek
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 421 psegttlrve dlvkqyfqta eknvqlsllt ergmgeavqe fvdkeekdai eelvkyqlek
 481 tqrflkerhi daledkidee vrrfretrqk ntneeddevr eamtraralr sqseesasaf
 541 saddlmsidl aeqmandsdd sisaatnkgr grgrgrrggr gqnsasrggs qrgradtgle
 601 tstrsrnskt avsasrnmsi idafkstrqq psrnvttkny sevievdesd veedifptts
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Schematic Representation of Mre11 Activity

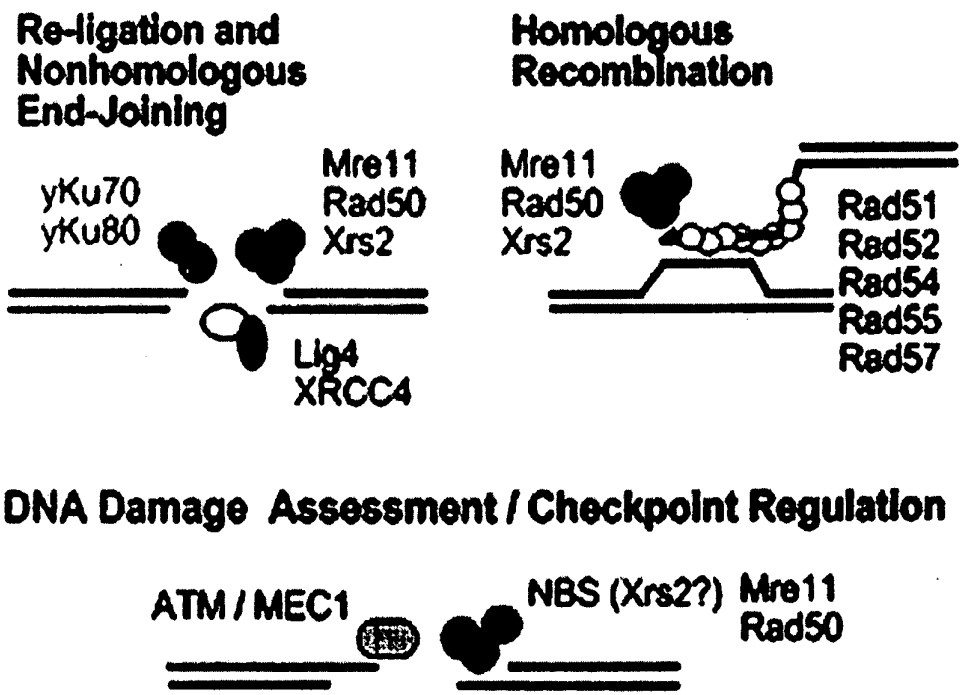


FIG. 3

Dominant Negative Mutants Generated for Target Validation Studies

Two inactivating mutants were generated analogous to catalytically inactiving mutations in the yeast MRE11:

H217Y (MCB1998 Jan;18(1):260-68)

H129N (MCB1999 Jan;19(1):556-66)

Both histidines are thought to form part of the Mn^{2+} coordination site (7 histidines coordinate 2 Mn^{2+} ions) in the catalytic core of the protein. H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA

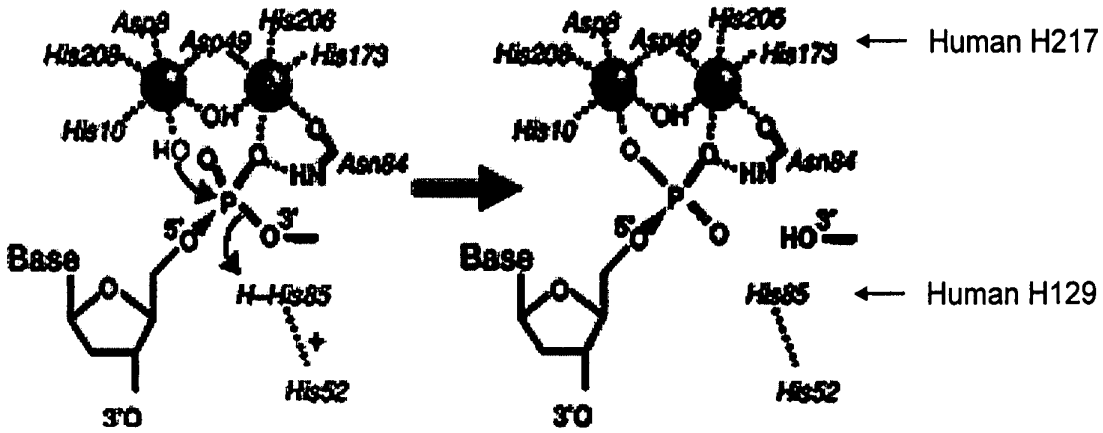
```
hMRE11      9  DENTFKILVATDIHLGFMEKDAARGNDTFVTLDEILRLAQENEVDFILLG GDLFHENKPS 68
               D +T +IL+ TD H+G+ E D   G+D++ T  E++ LA+ N VD ++   GDLFH NKPS
SCMRE11     5  DPDTIRILITTDNHVGYNENDPITGDDSWKTFHEVMMLAKNNNVDMVVQSGDLFHVNKPS 64

           69  RKTLHTCLELLRKYCMGDRPVQFEILSDQSVNFGFSKFPWVNYQDGNLNISIPVFSIHGN 128
               +K+L+  L+ LR  CMGD+P + E+LSD S  F + +F  VNY+D N NI SIPVF I GN
           65  KKSLYQVLKTLRLCCMGDKPCELELLSDPSQVFHYDEFTNVNYEDPNFNISIPVFGISGN 124
               *
          129  HDDPTGADALCALDILSCAGFVNHFGRSMSVEKIDISPVLLQKGSTKIALYGLGSIPDER 188
               HDD +G   LC +DIL   G +NHFG+ +  +KI + P+L QKGSTK+ALYGL ++ DER
          125  HDDASGDSLLCPMDILHATGLINHFGKVIESDKIKVVPLLFQKGSTKLALYGLAAVRDER 184
                                          *
          189  LYRMFVNKKVTMLRPKEDENSWFNLFVIHQNRSKHGSTNFIPEQFLDDFIDLVIWGHEHE 248
               L+R F +  VT   P   E  WFNL  +HQN + H +T F+PEQFL DF+D+VIWGHEHE
          185  LFRTFKDGGVTFEVPTMREGEWFNLMCVHQNHTGHTNTAFLPEQFLPDFLDMVIWGHEHE 244

          249  CKIAPTKNEQQLFYISQPGSSVVTSLSPGEAVKKHVGLLRIK-GRKMNMHKIPLHTVRQF 307
               C      N  + F + QPGSSV TSL   EA  K+V +L IK G    M  IPL T+R F
          245  CIPNLVHNPIKNFDVLQPGSSVATSLCEAEAQPKYVFILDIKYGEAPKMTPIPLETIRTF 304
```

FIG. 4

Representation of Active Site of *P. furiosus* Mre11



Mn^{2+} coordination site 7 histidines coordinate 2 Mn^{2+} ions in the catalytic core of the protein.

H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA

Mutations Generated for Dominant Negative Studies correspond to:

P. furiosus	***Human***
H173	H217
H85	H129

FIG. 5

Summary of Target Validation Studies: MRE11

Dominant negative studies

	Antiproliferative Activity						
	Tumor				Normal		
	A549	Hela	PC3	H1299	HMEC	HUVEC	PrEC
Wt							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
H217Y							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
H129N							
GFP-fusion	++	++	-/+	-/+	-	-	-
IRES GFP	+	-	nd	nd	-	-	nd

Antisense: **A549 inconclusive**

(+ indicates antiproliferative effect in either the GFP positivity study, cell tracker or antisense studies)

FIG. 6

Summary of Target Validation Studies: MRE11

Dominant negative studies

	Chemosensitization Activity		
	Tumor		
	A549	Hela	HMEC
Wt			
GFP-fusion	-	-	-
H217Y			
GFP-fusion	++	++	-

FIG. 7

Overexpression of GFP- Fused MRE11 H129N is Antiproliferative in A549 and Hela Cells

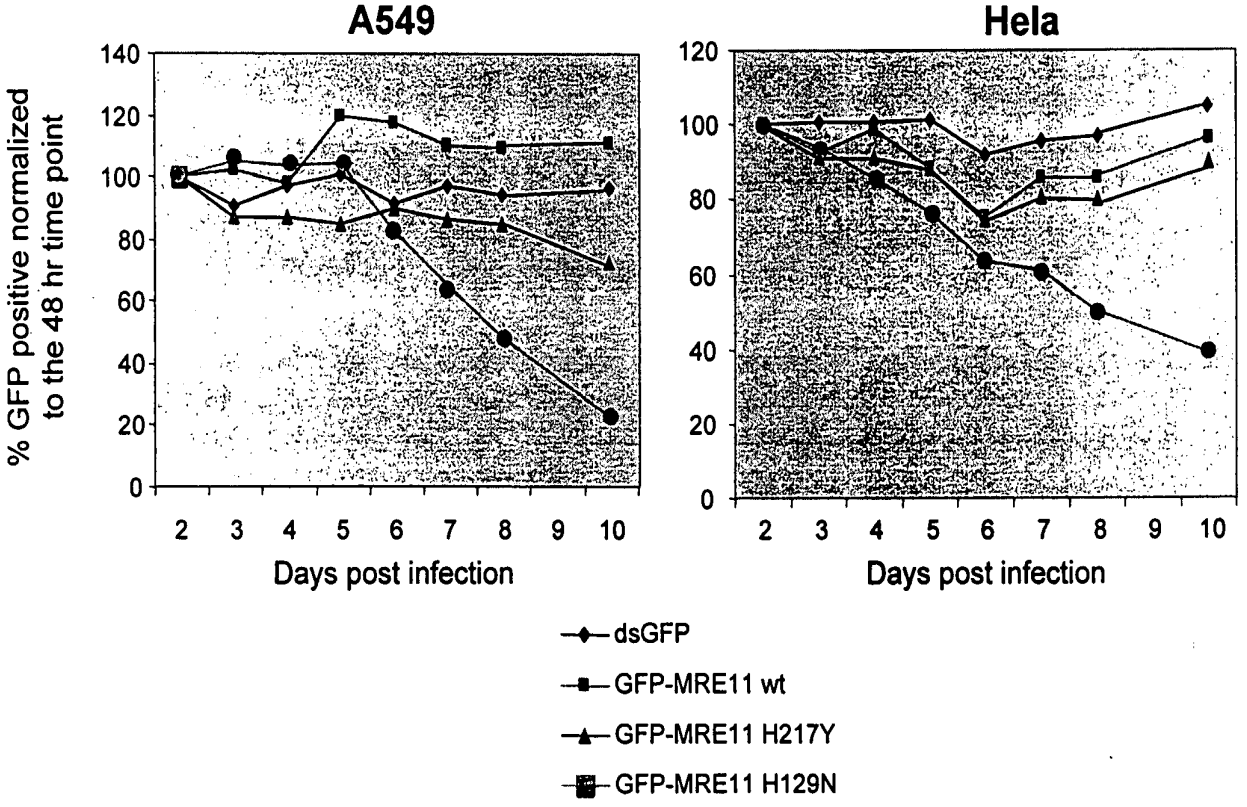
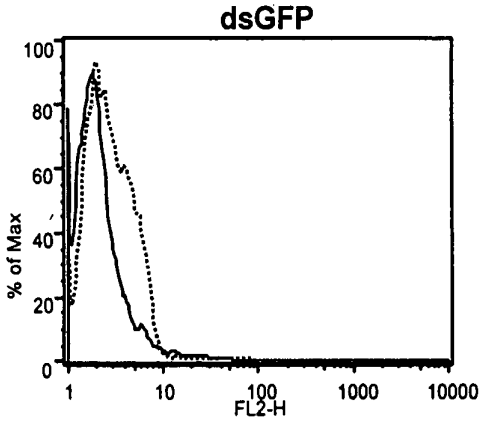


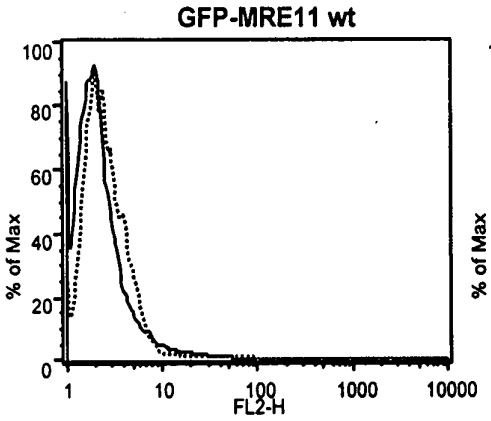
FIG. 8

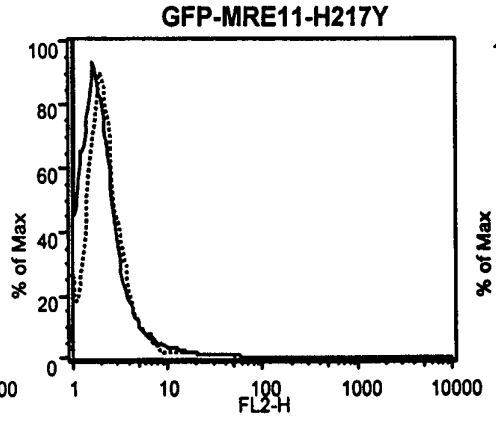
Cell Tracker Assay Shows the Activity of GFP-fused MRE11 H129N is Antiproliferative in A549 Cells



Cell tracker analysis 9 days post infection

Dotted = GFP positive
Solid = GFP negative





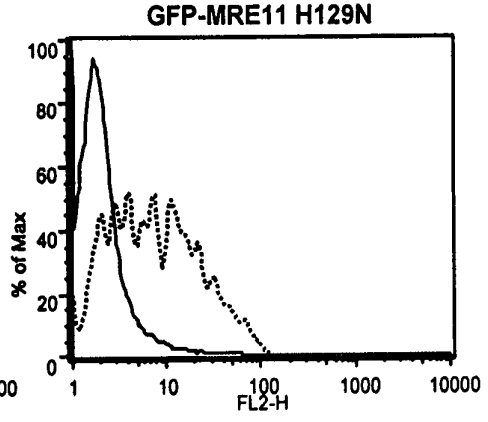
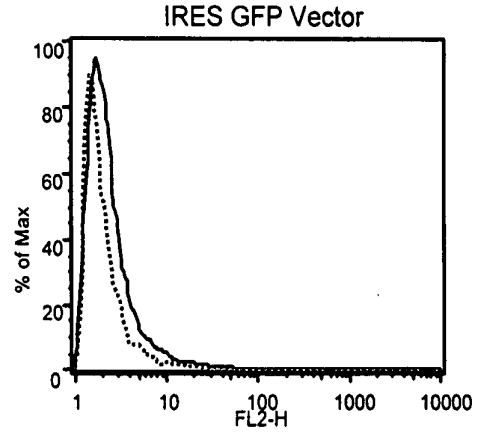


FIG. 9

Cell Tracker Assay Shows the Activity of MRE11 H129N Using IRES GFP is Antiproliferative in A549 Cells



Cell tracker analysis 9 days post infection

Dotted = GFP positive
Solid = GFP negative

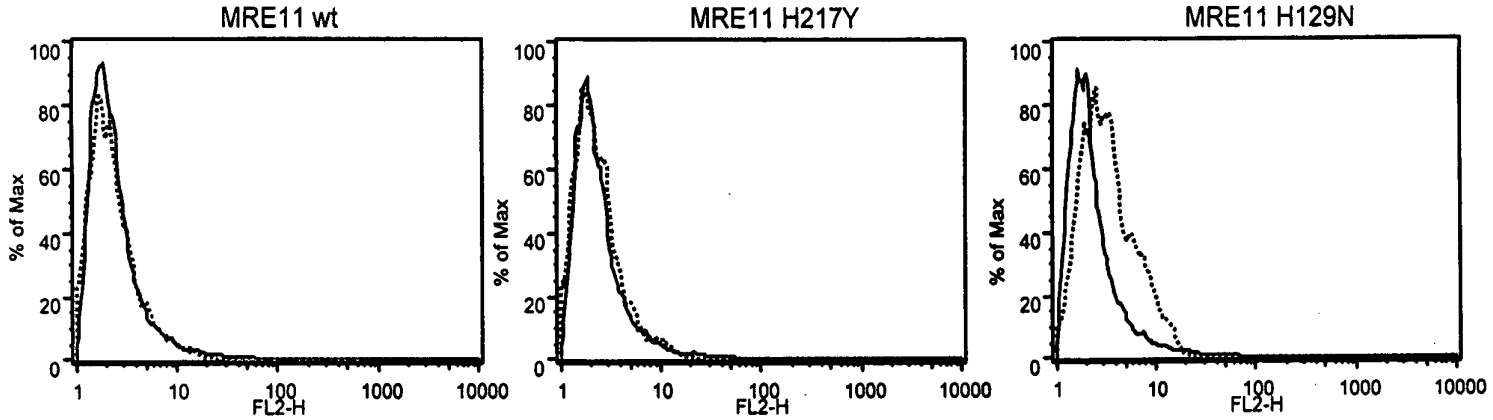


FIG. 10

Overexpression of GFP-fused MRE11 Wild Type and Mutants in PC-3 and H1299 cells

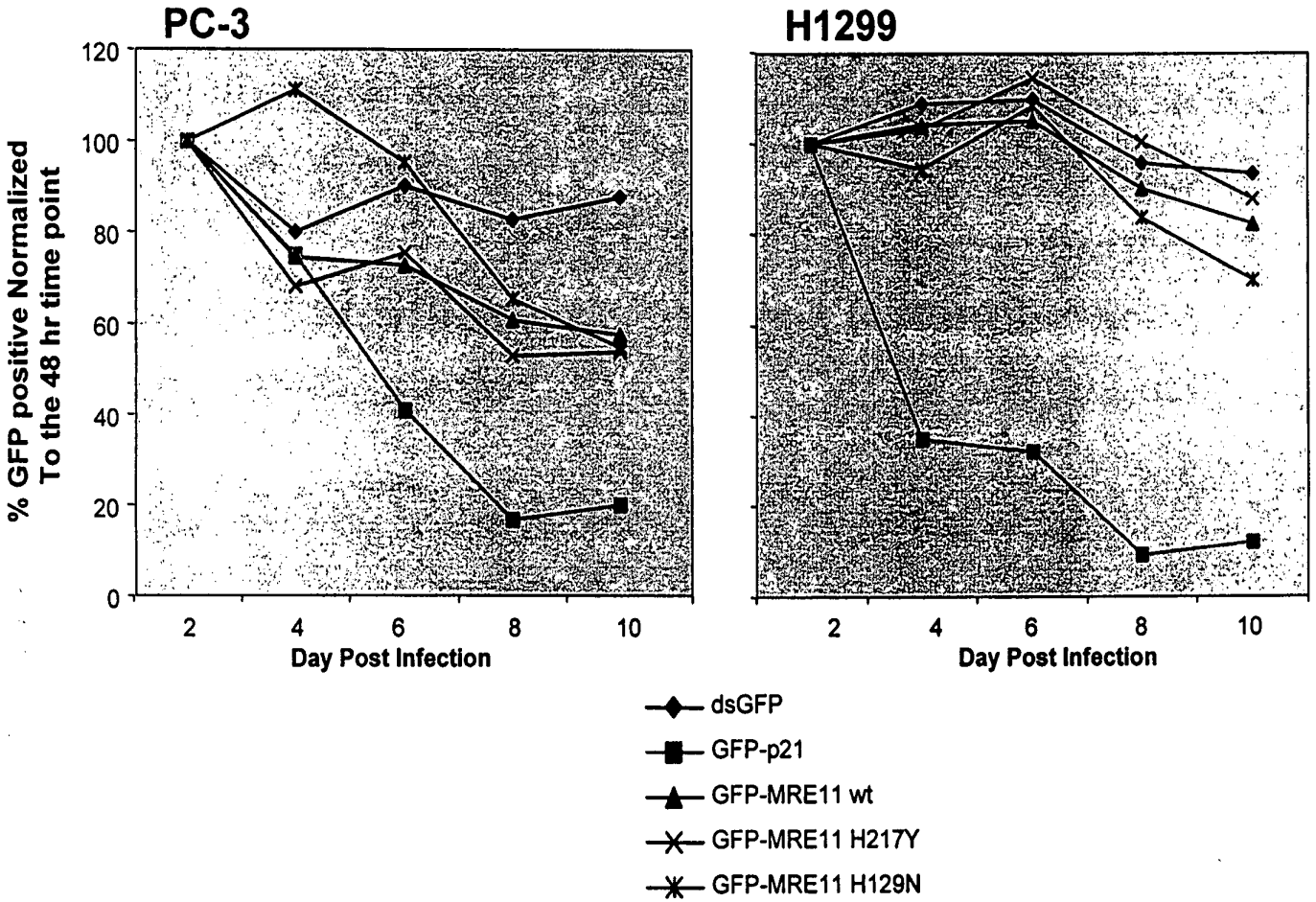


FIG. 11

Overexpression of MRE11 Wild Type and Mutants is Not Antiproliferative in Normal Cells

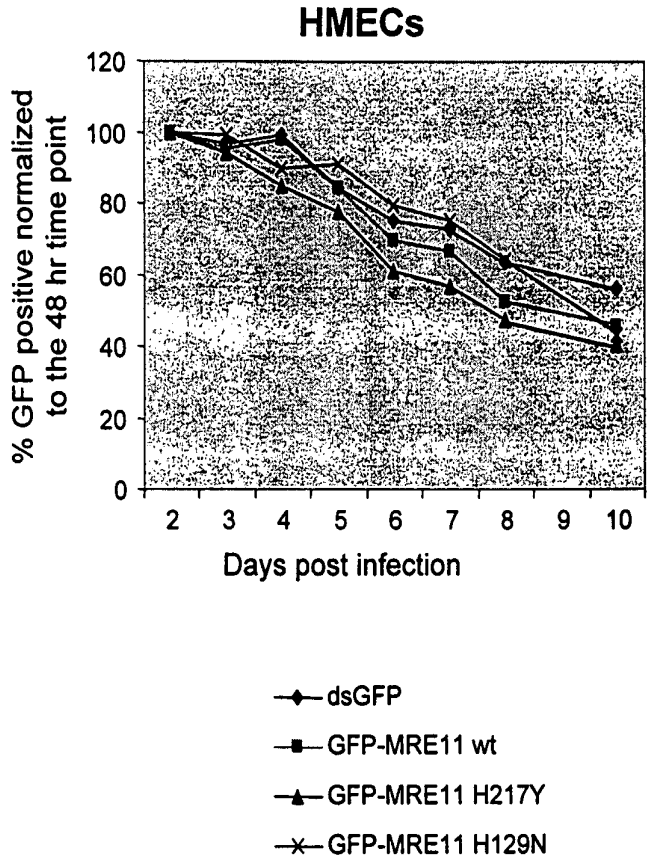
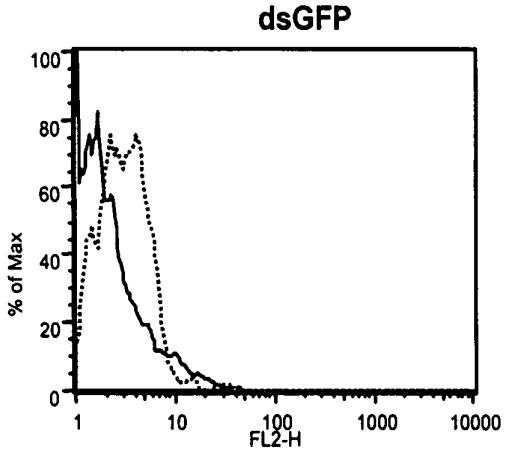


FIG. 12

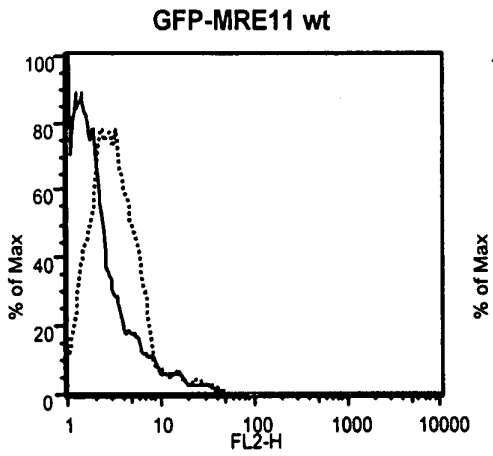
No Antiproliferative Activity of MRE11 Wild type or Mutant Proteins is Detected By the Cell Tracker Assay in Normal Cells

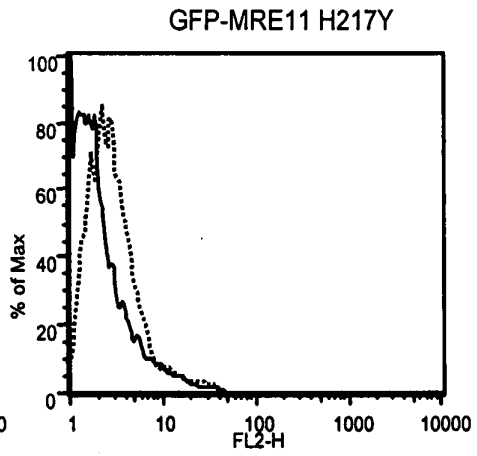


HMECs

Cell tracker analysis 10 days post infection

Dotted = GFP positive
Solid = GFP negative





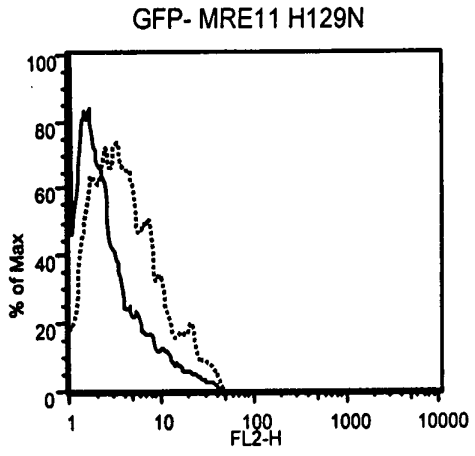


FIG. 13

Overexpression of MRE11 Wild Type and Mutants is Not Antiproliferative in Normal Cells

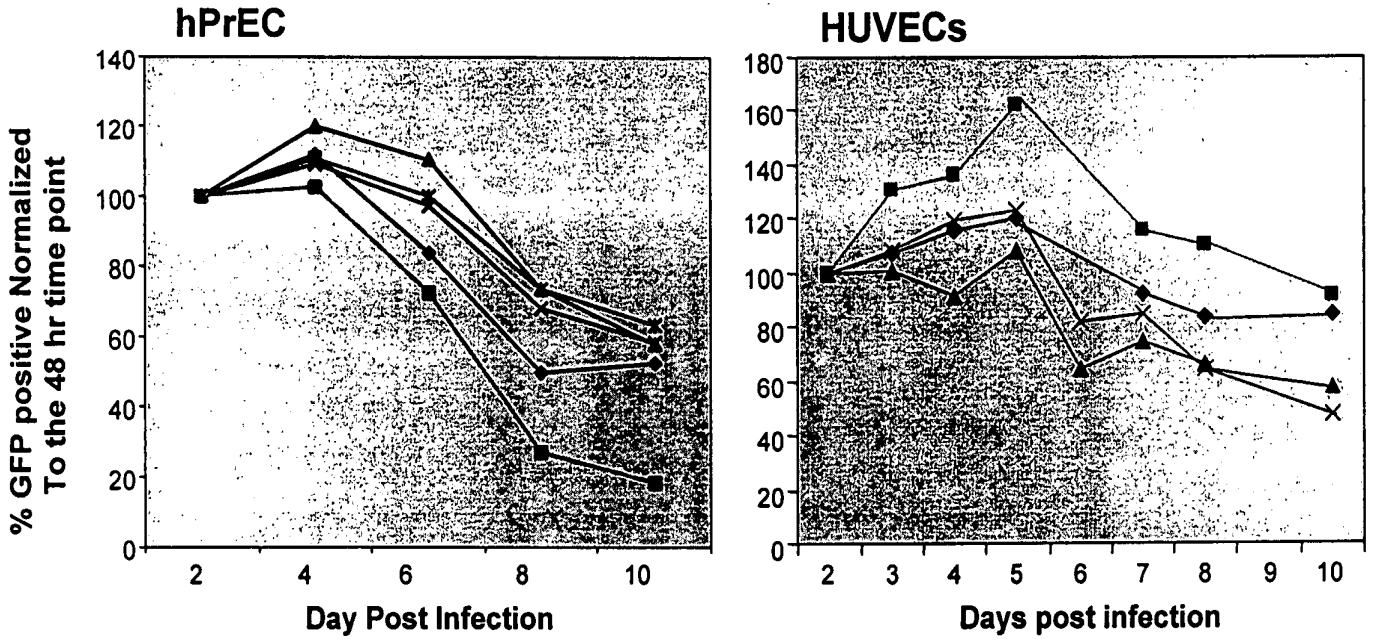


FIG. 14

MRE11 Specific Antisense Oligo Effects in A549 Cells

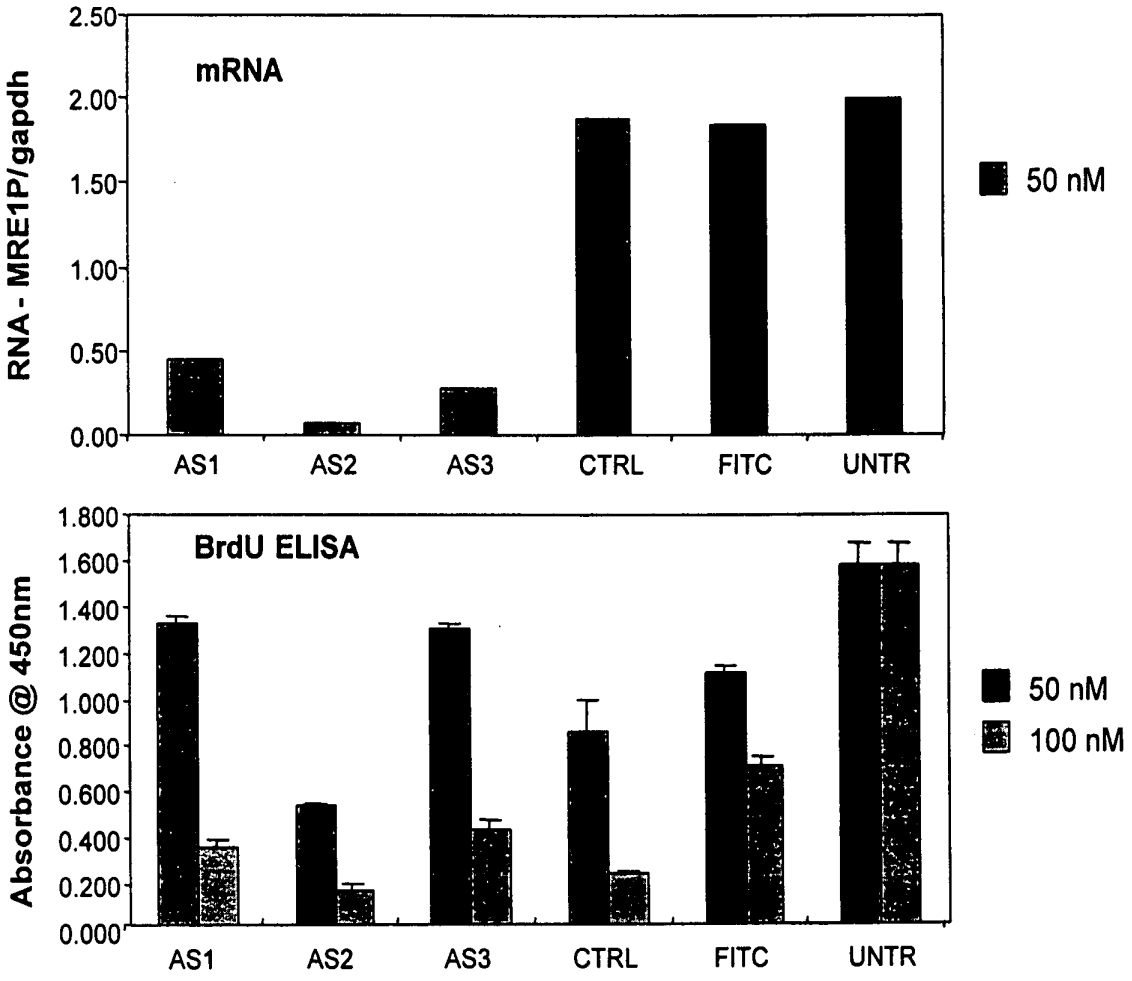


FIG. 15

Strategies for Assessing Chemosensitization Using Dominant Negative Studies

Plate based BrdU incorporation ELISA

Hela cells were infected with GFP-fused wt or mutant MRE11

The top 10% GFP positive cells were sorted 5 days after infection

Purified cell populations were plated in 96-well plates for chemotherapeutic treatments

BrdU incorporation was measured 48 and 72 after treatment

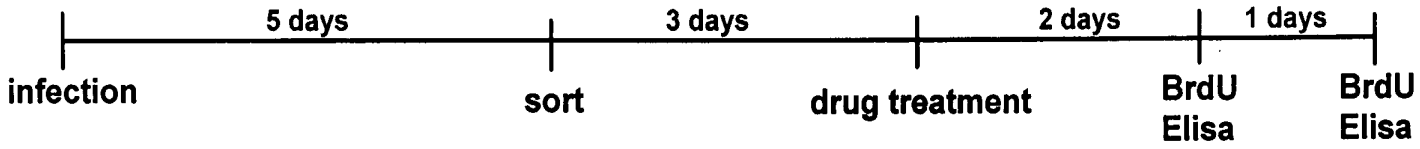


FIG. 16

Plate-based Chemosensitization Studies of Sorted HeLa Cells Expressing GFP-fused Wild Type or Mutant MRE11

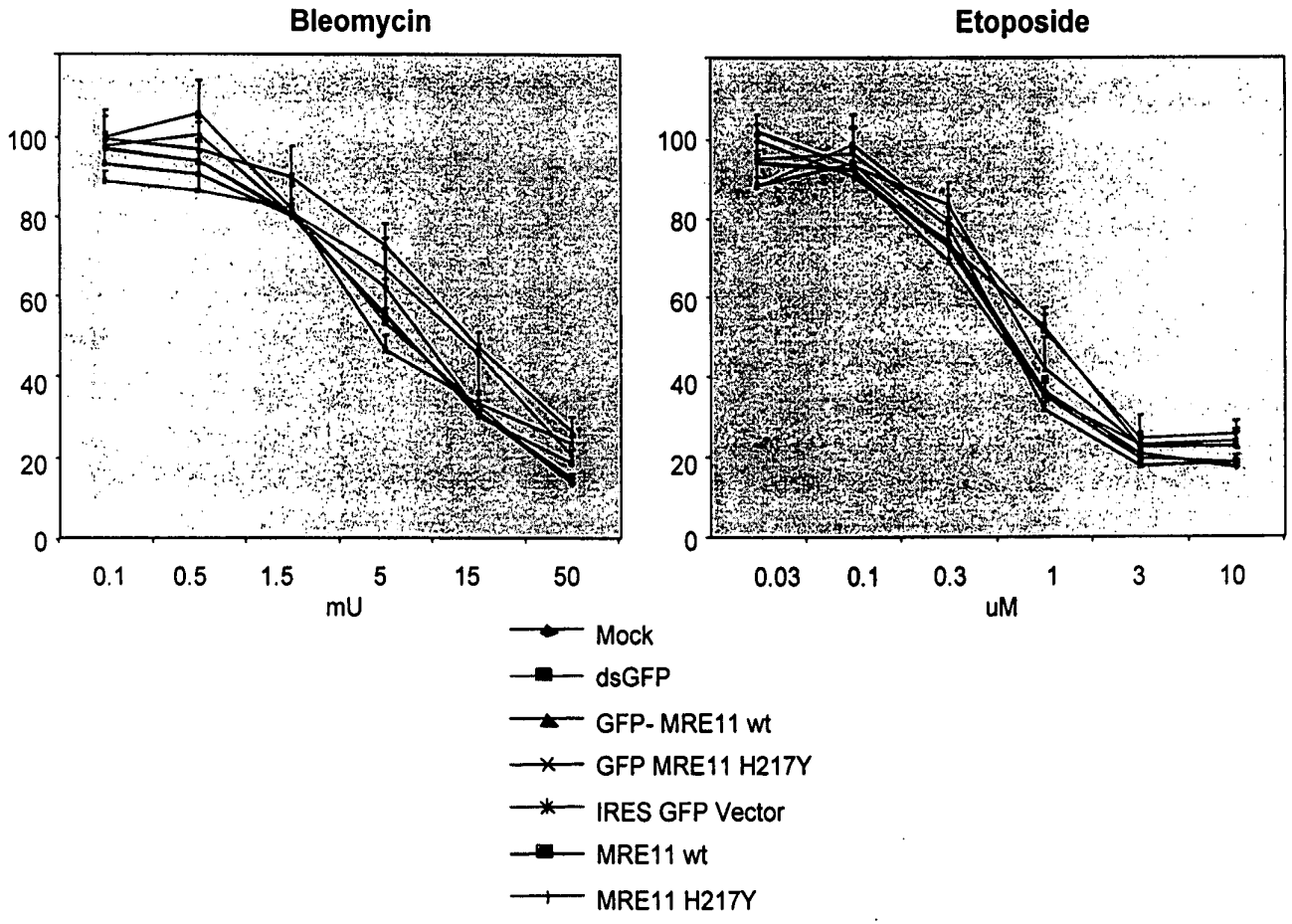


FIG. 17

Alternative Strategy for Assessing Dominant Negative Chemosensitization Effects

C II Survival after Drug treatment and wash out (similar to col ony survival assay)

GFP positivity study to determine selective sensitivity of the GFP positive cells (reflecting cells expressing wild type or mutant proteins) in a mixed population of infected and non-infected cells

- Cells are infected with GFP-fused wt or mutant MRE11
- Cells are then treated with chemotherapeutics for 48 hrs
- After a 48 hr treatment period, the chemotherapeutic is washed out and cells are allowed to recover for 5-8 day.
- After the end of the recovery period, the %GFP positive cells in the treated population relative to the untreated population is assessed by FACS analysis.

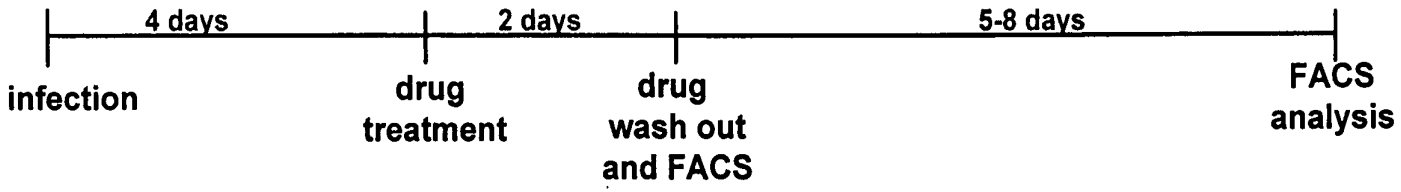


FIG. 18

No Selective Sensitization of MRE11 Wild Type or Mutant Expressing A549 Cells 48 hrs After Bleomycin Treatment

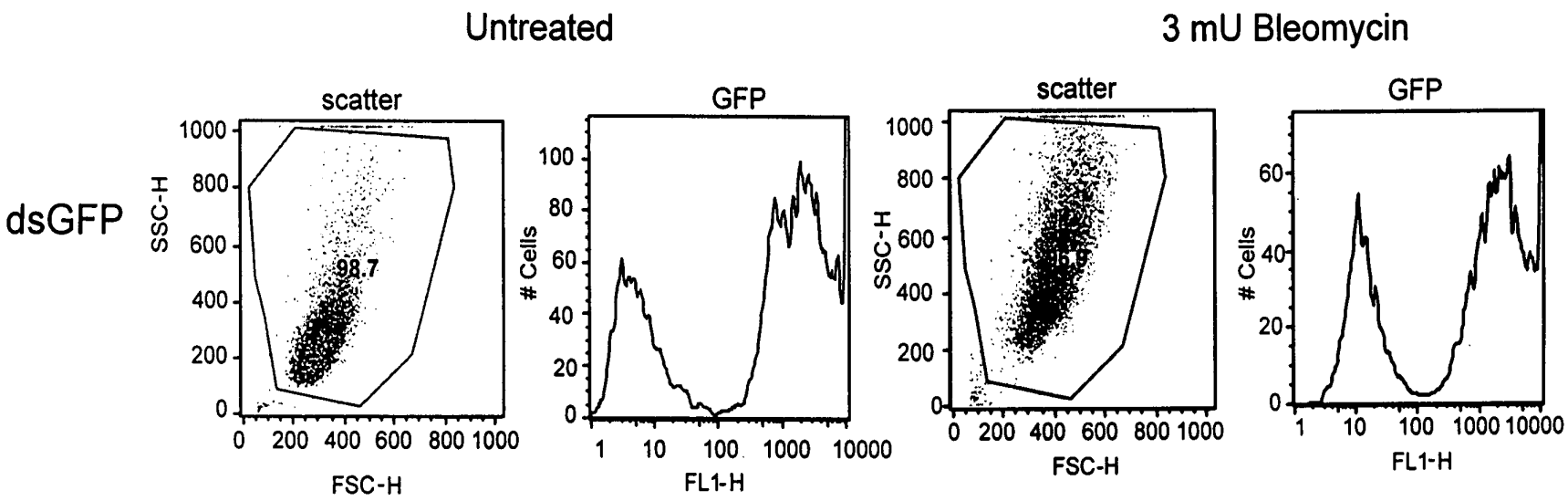


FIG. 19A

No Selective Sensitization of MRE11 Wild Type or Mutant Expressing A549 Cells 48 hrs After Bleomycin Treatment (cont'd)

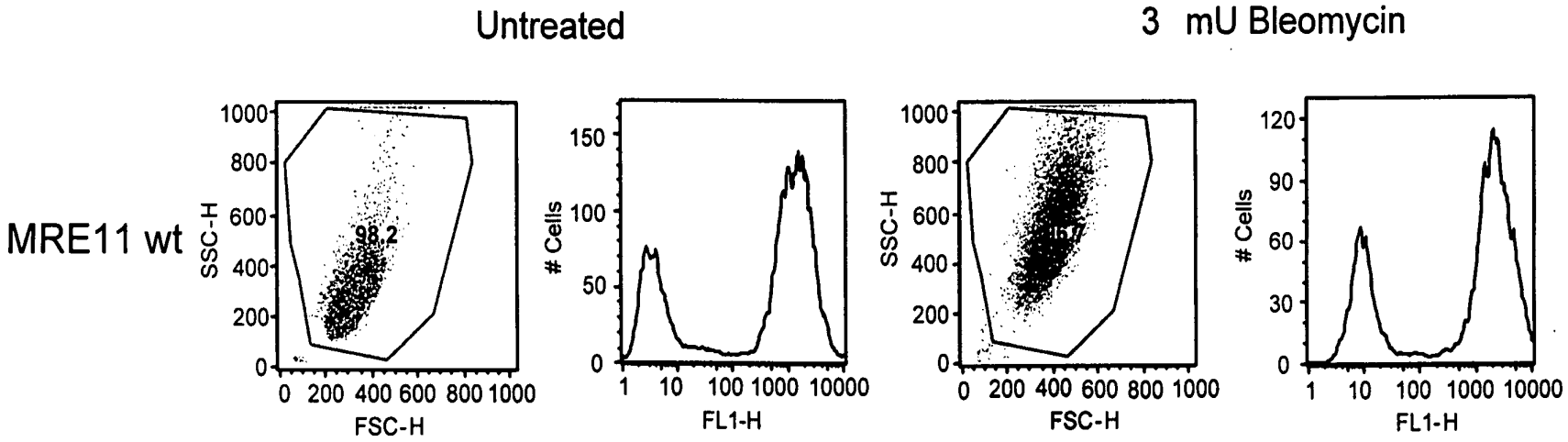


FIG. 19B

No Selective Sensitization of MRE11 Wild Type or Mutant Expressing A549 Cells 48 hrs After Bleomycin Treatment (cont'd)

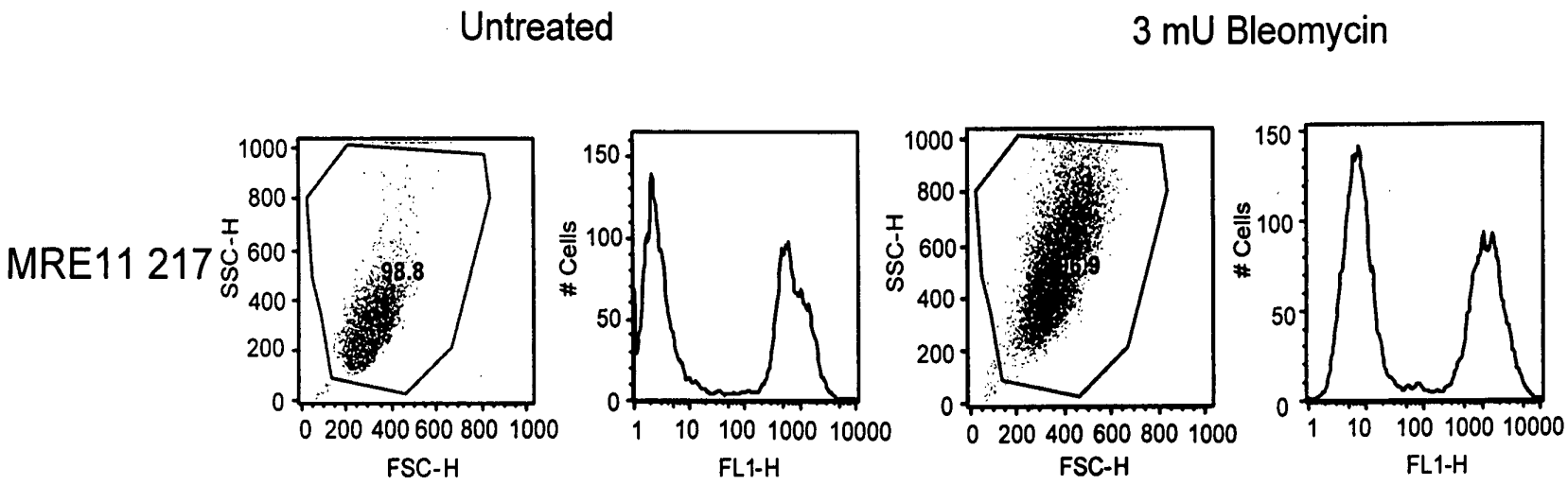


FIG. 19C

A549 Cells Expressing the MRE11 H217Y Mutant Fail to Recover From Bleomycin Treatment

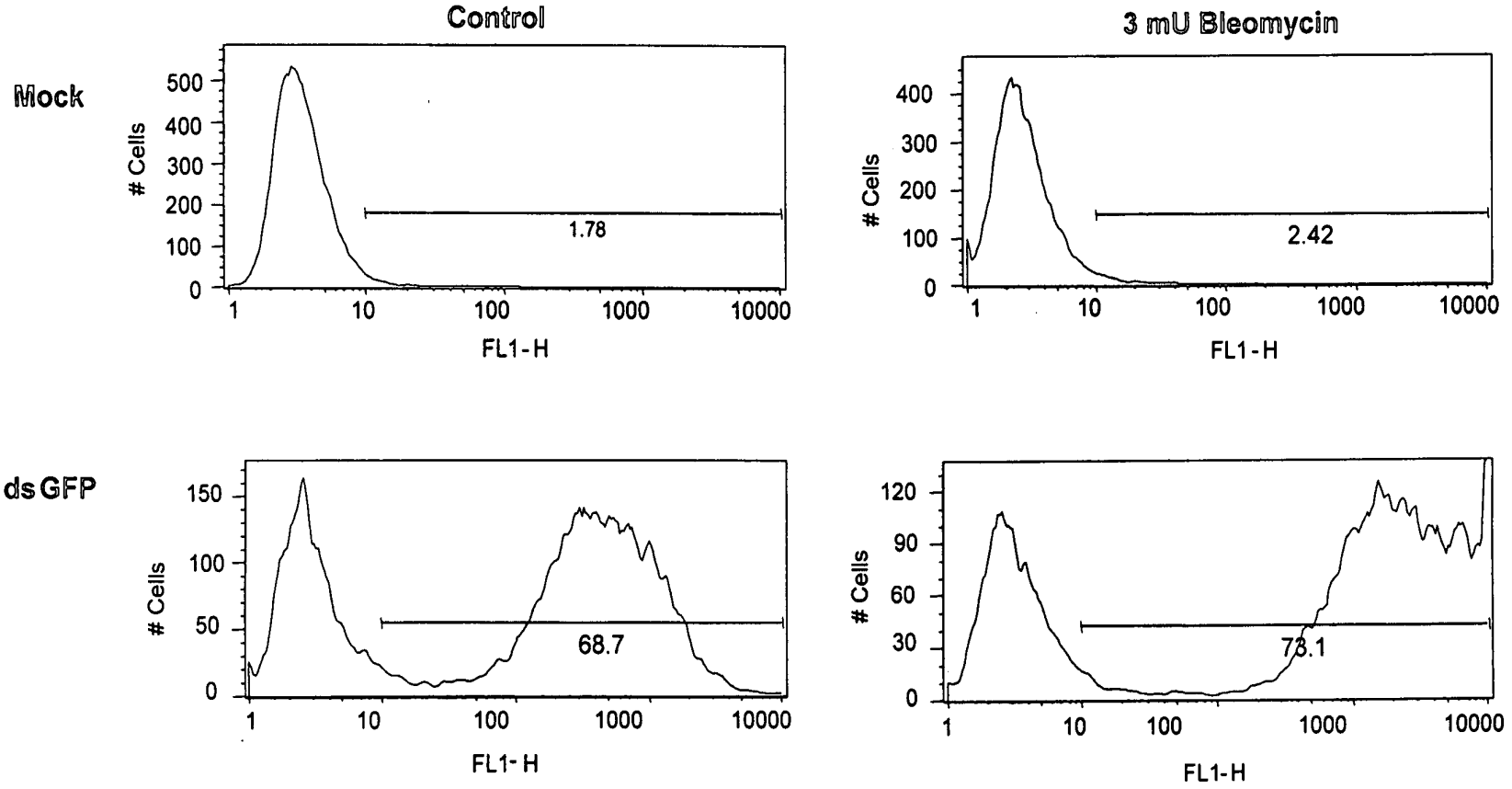


FIG. 20A

A549 Cells Expressing the MRE11 H217Y Mutant Fail to Recover From Bleomycin Treatment (cont'd)

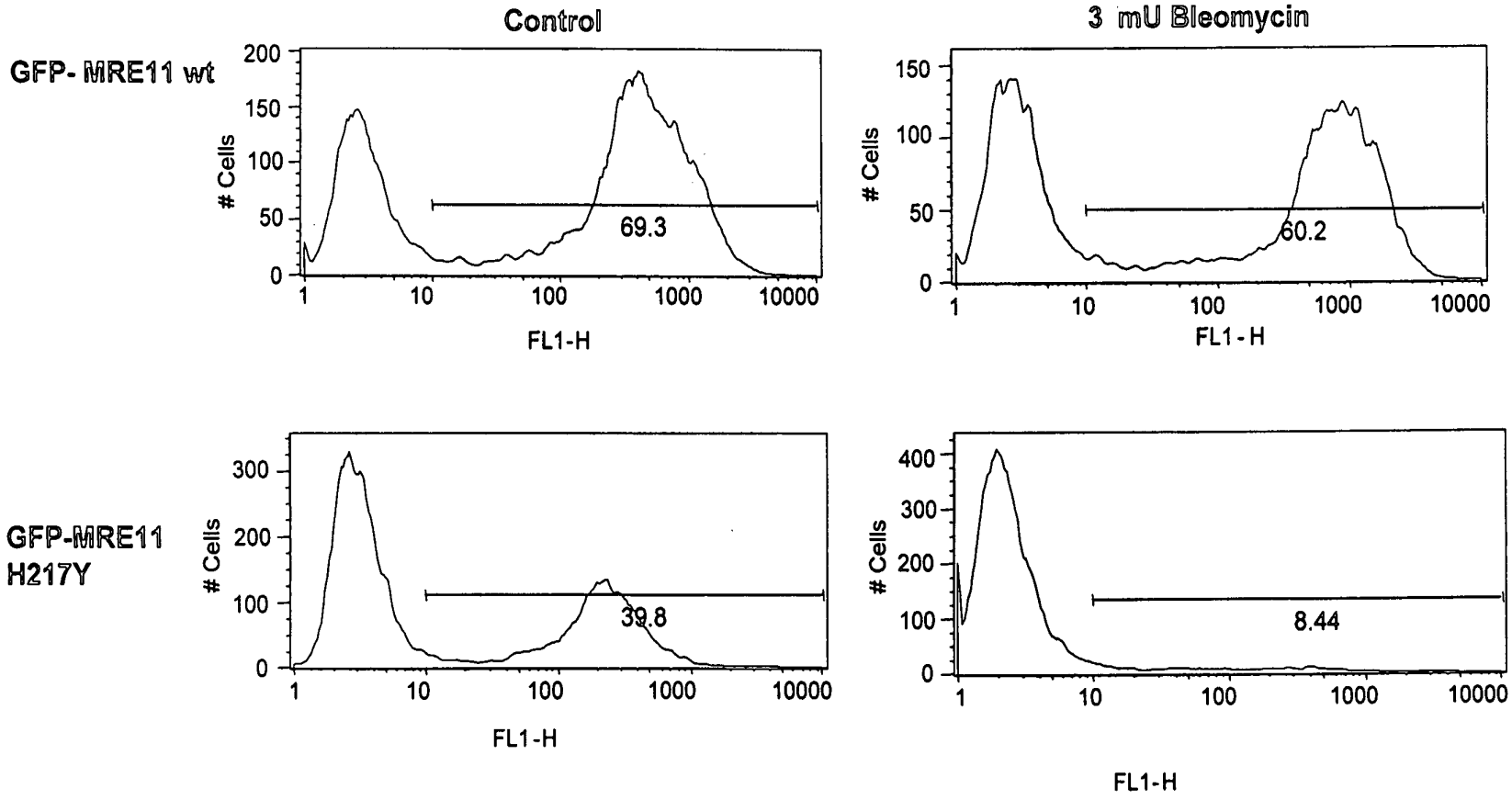


FIG. 20B

Overexpression of GRP-MRE11 H217Y Mutant in A549 and HeLa Cells Enhances Sensitivity to Bleomycin Treatment

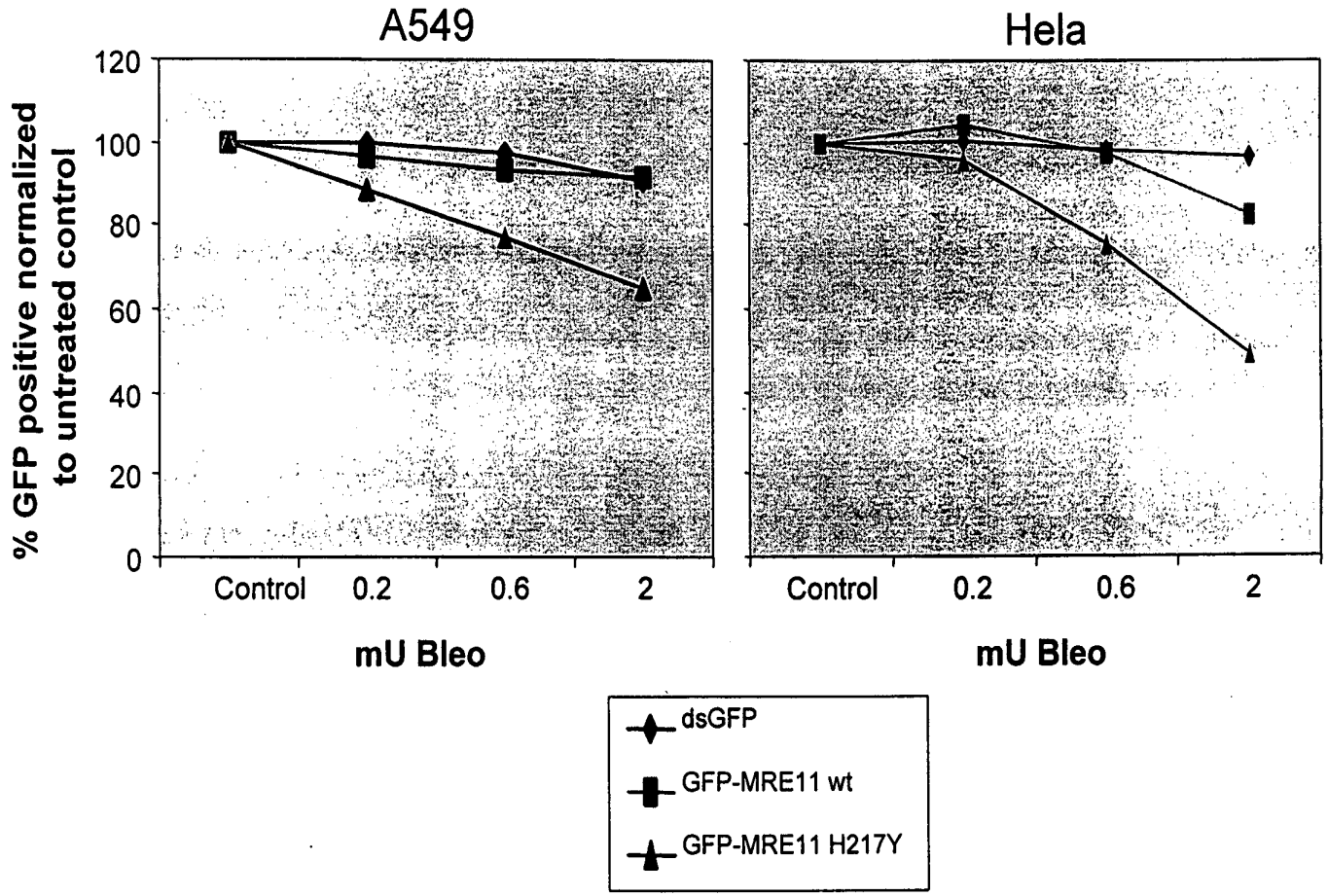


FIG. 21

Overexpression of GFP-MRE11 H217Y Mutant in Normal HMECs does not Enhance Sensitivity to Bleomycin Treatment

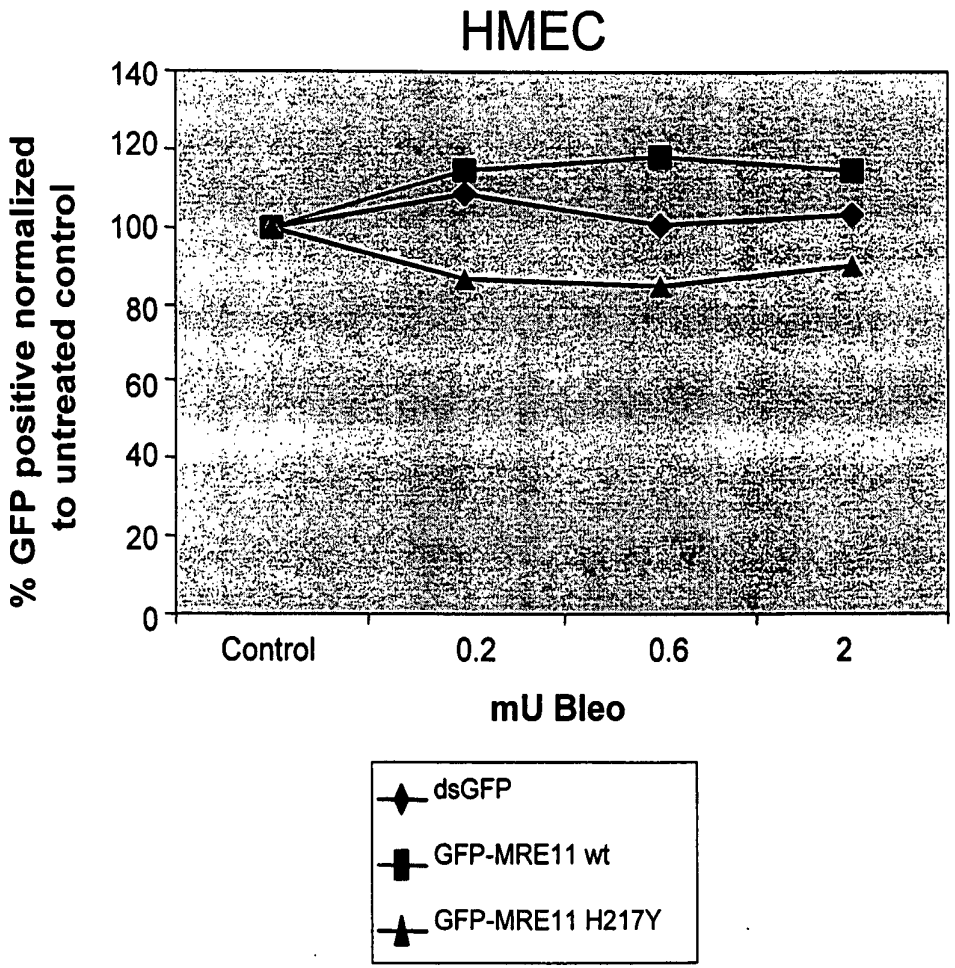
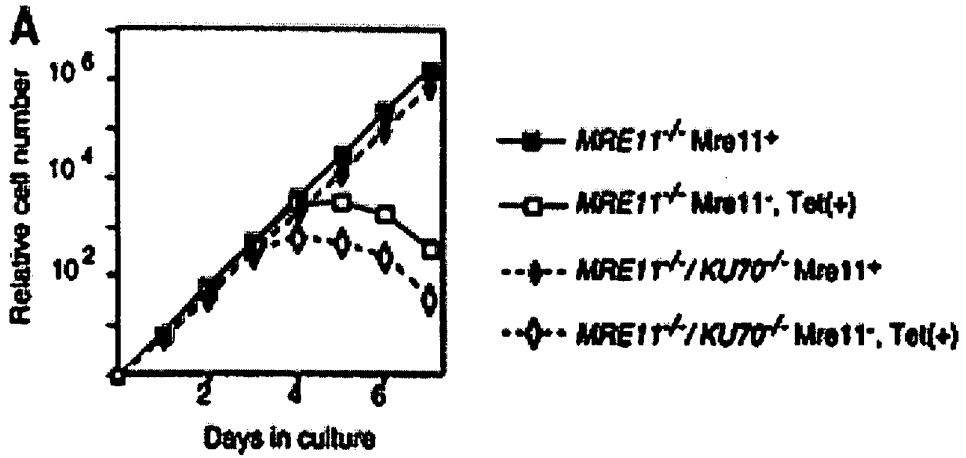


FIG. 22

Depletion of MRE11 is Antiproliferative in the Hyper-recombinogenic Chicken B-cell line DT40 Made Conditionally Null for MRE11



Additional phenotypes observed In MRE -/- cells

- chromosomal aberrations
- centrosome amplification
- enhanced sensitization to ionizing radiation

EMBO J. 1999 Dec 1;18(23):6619-29.

FIG. 23

Possible Models Explaining the Antiproliferative and Chemosensitization Effects of MRE11 Inhibition

Antiproliferative activity may be explained through MRE11's Role in:

Double strand break repair

Telomeric regulation

FIG. 24

MRE11 Inhibition May Block Repair of Spontaneous Or Drug Induced Double Strand Breaks

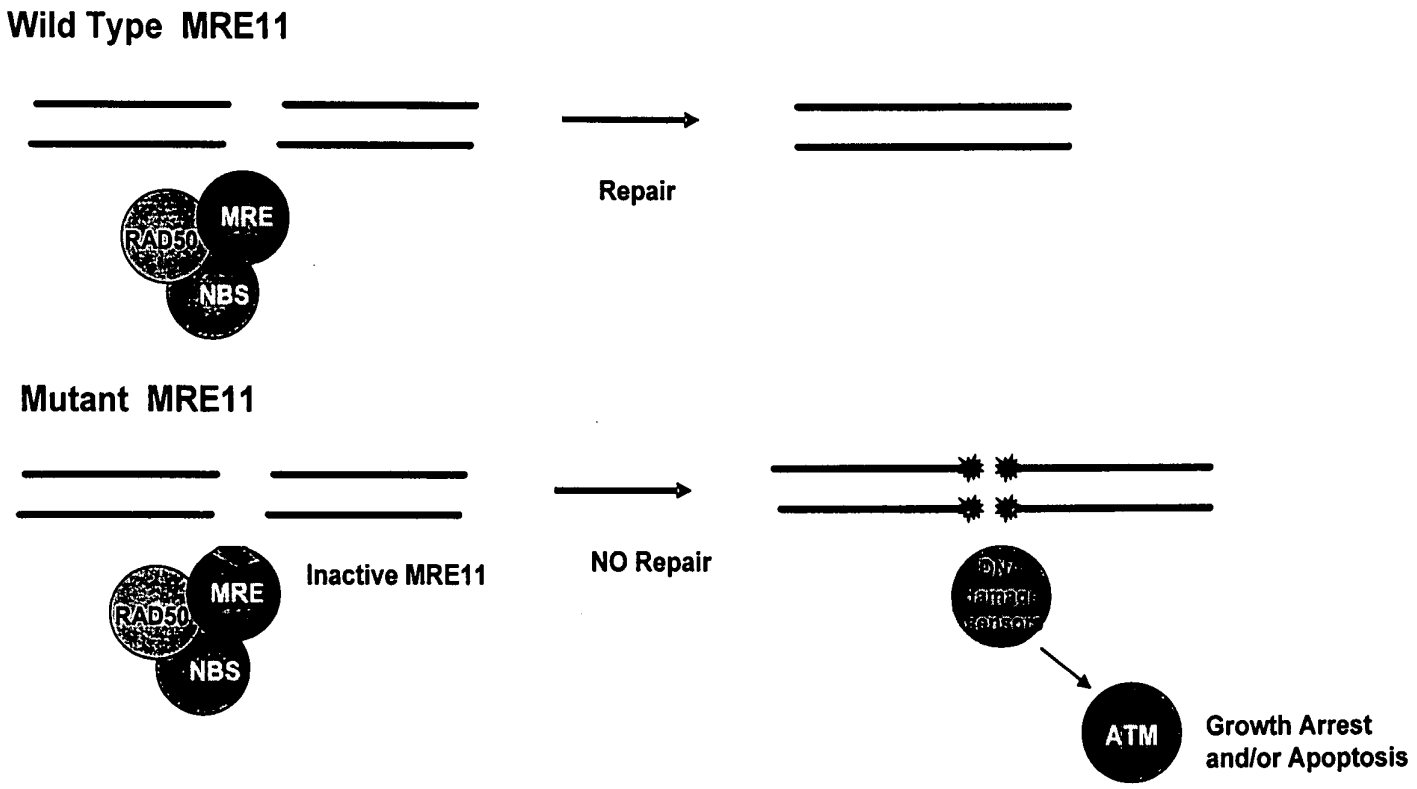


FIG. 25

MRE11 Inhibition May Block the Formation of the Protective T-loop Structure at Telomere Ends

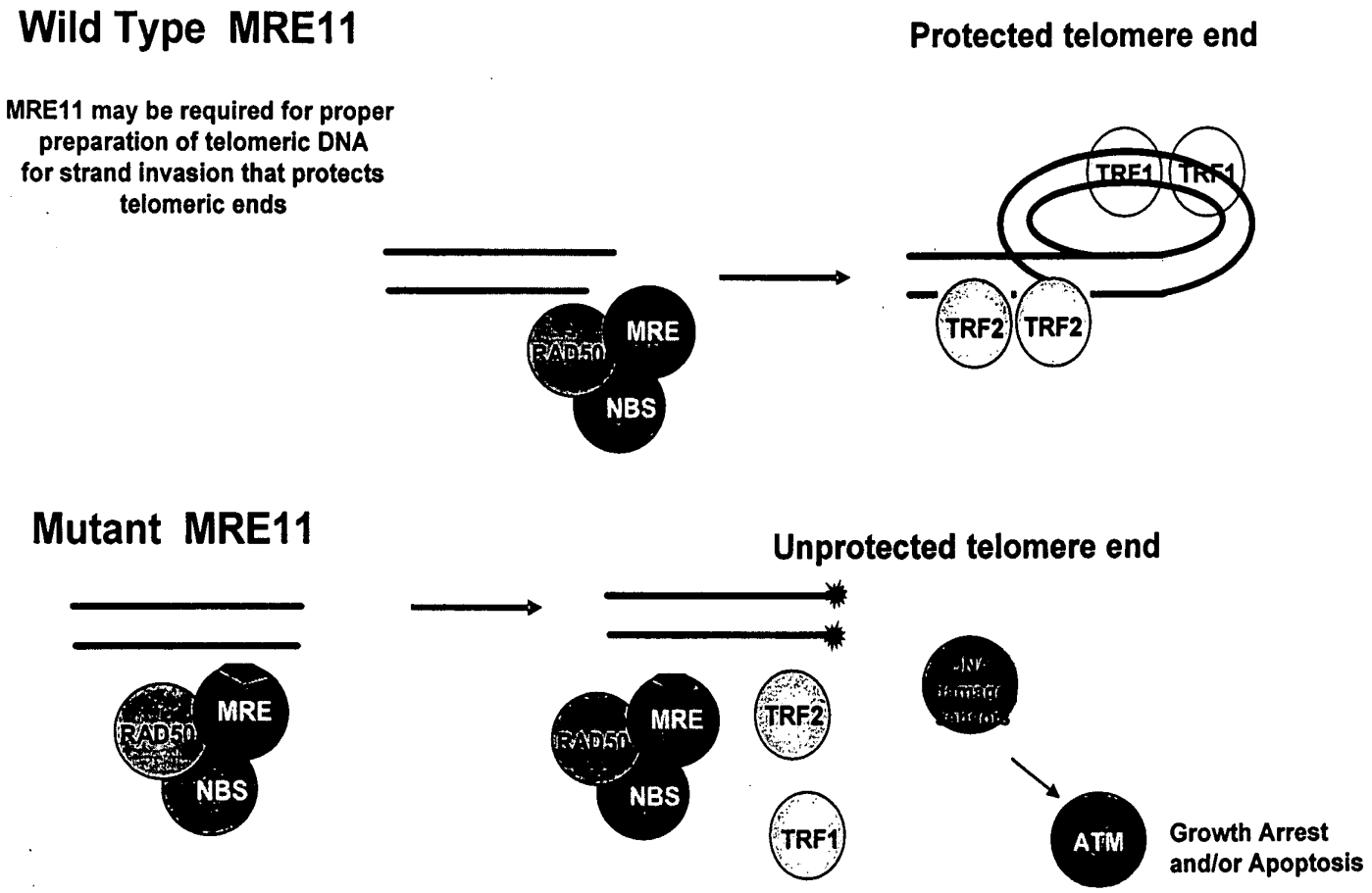


FIG. 26

MRE11 Summary

Functional Studies **Source: YTH- PCNA/Nbs1**

Antiproliferative Activity

- Overexpression of MRE11 H129N mutant protein is antiproliferative in tumor cells, but not in normal cells
- No strong antiproliferative effect is seen in cells expressing MRE11 wild type or H217Y mutant

Chemosensitization

- Overexpression of MRE11 H217Y mutant enhances sensitivity to chemotherapeutic treatment in tumor cells
- Sensitization by the H129N mutant cannot be assessed because of the inherent antiproliferative activity seen with expression of this mutant

Literature

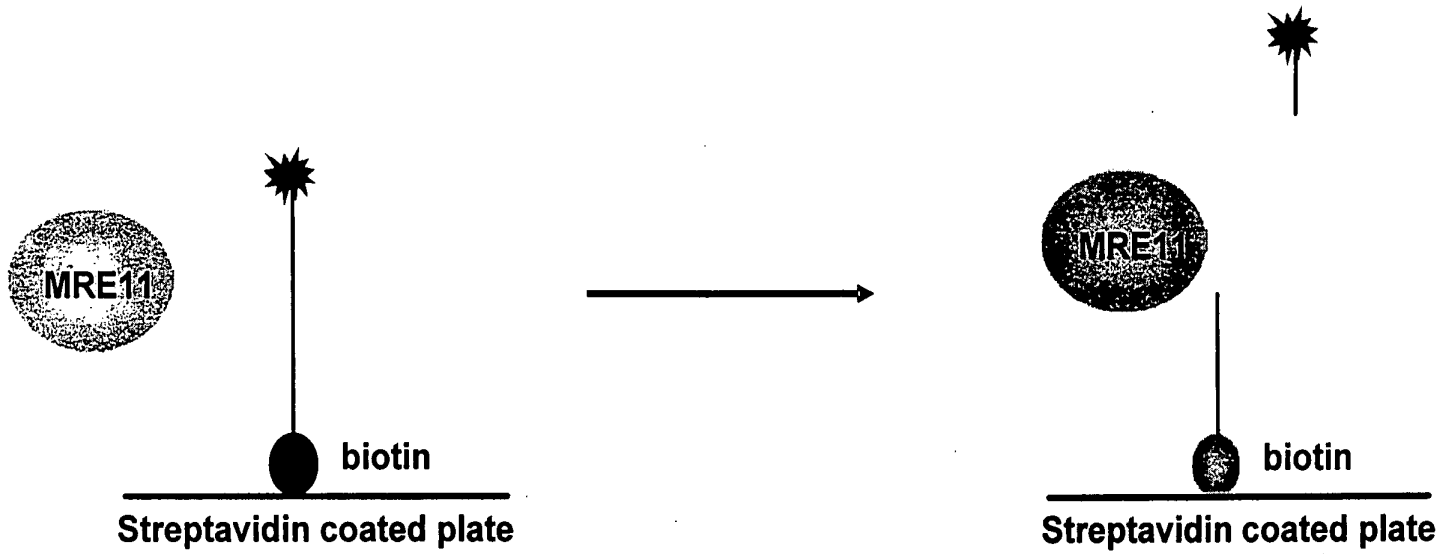
- Numerous studies have suggested that MRE11 plays an important role in DNA damage repair pathway
- Studies on the yeast protein suggest that inhibition of catalytic activity of MRE11 will result in sensitivity to ionizing radiation

Conclusion

- Functional studies suggest inhibition of MRE11 will selectively inhibit tumor cell growth and enhance the response of tumor cells to DNA damaging agents

FIG. 27

Proposed HTS Compatible Biochemical Assay for MRE11



Fluorescently labeled ssDNA substrate

Endo/exonuclease Activity is measured as loss of fluorescence

FIG. 28

Oligonucleotide Duplex Substrate for MRE11 Plate-Based Assay

Sequence was taken from oligonucleotide DG51 (Paul and Gellert, Mol. Cell, 1998),a substrate used to characterize the *in vitro* nuclease activity of recombinant Mre11. A *HaeIII* cleavage site was incorporated as a positive control for the assay.

FIG. 29

Biochemical Assay for MRE11 Exonuclease Activity

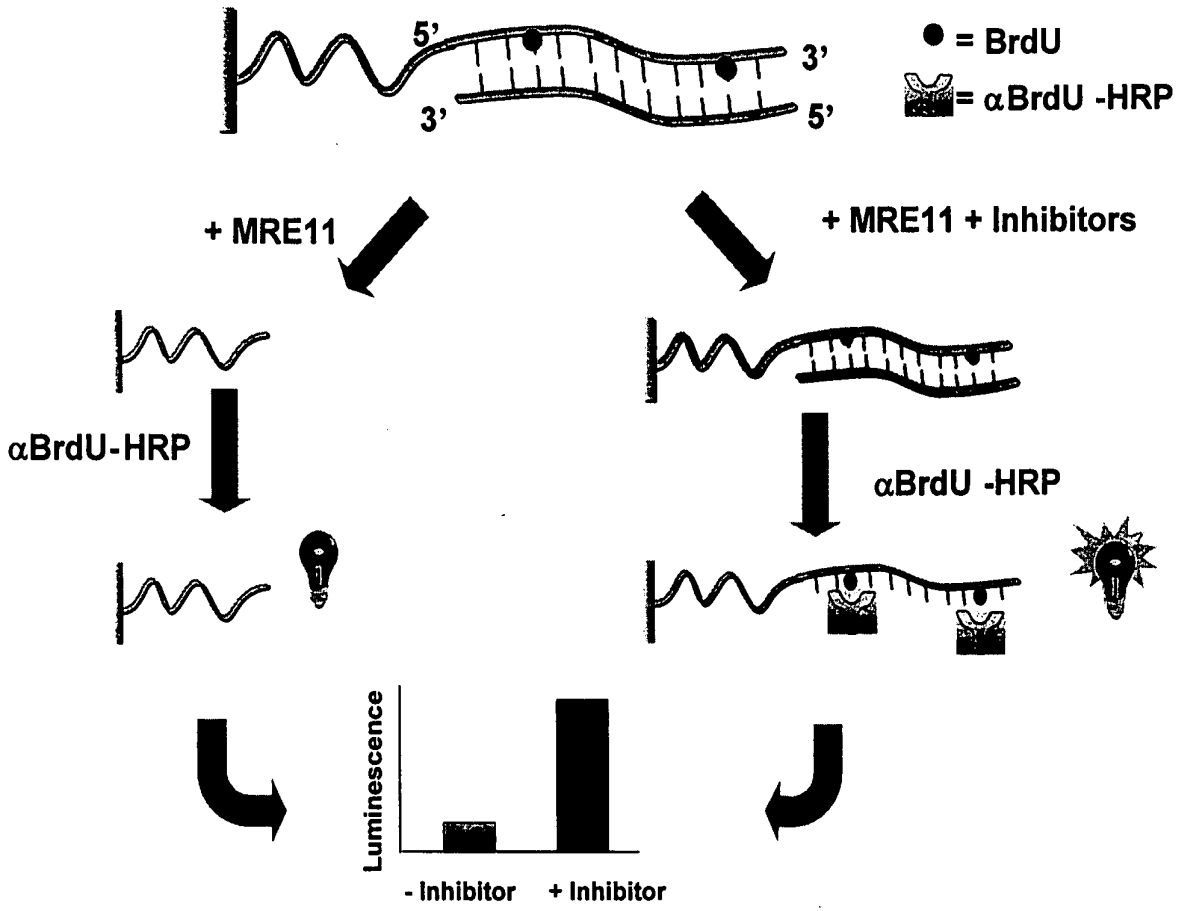


FIG. 30

Cleavage of Single-stranded Biotinylated Reporter Oligonucleotide by MRE11

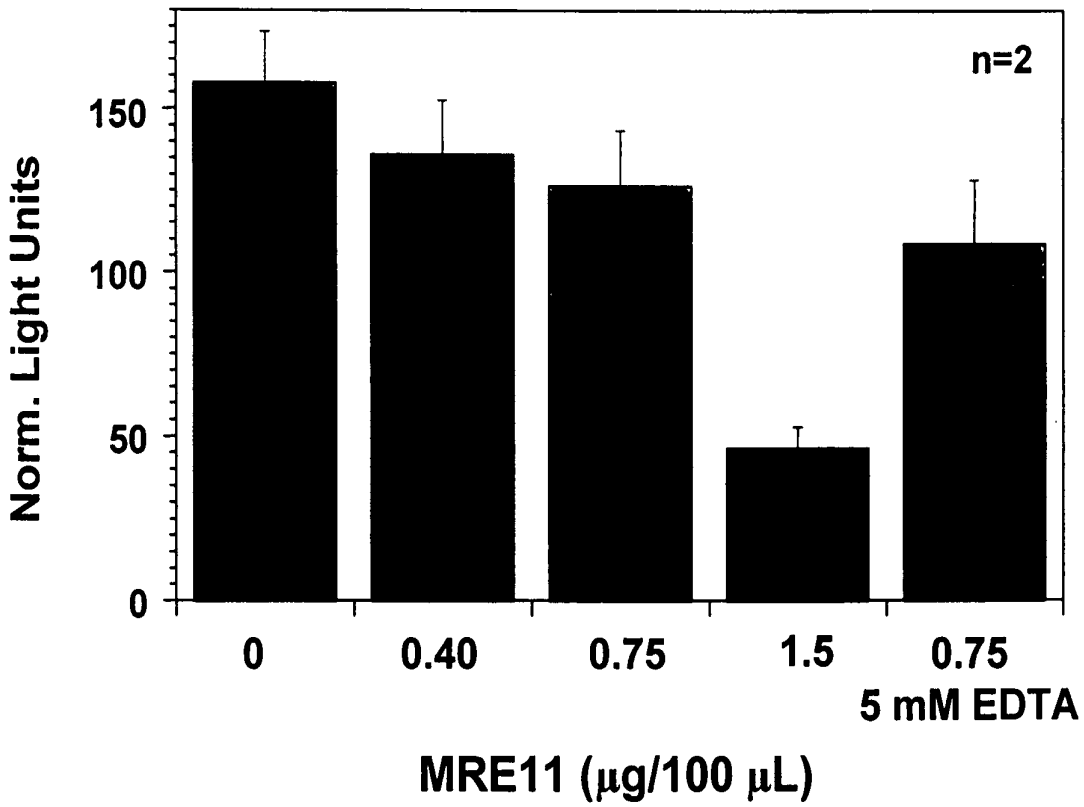
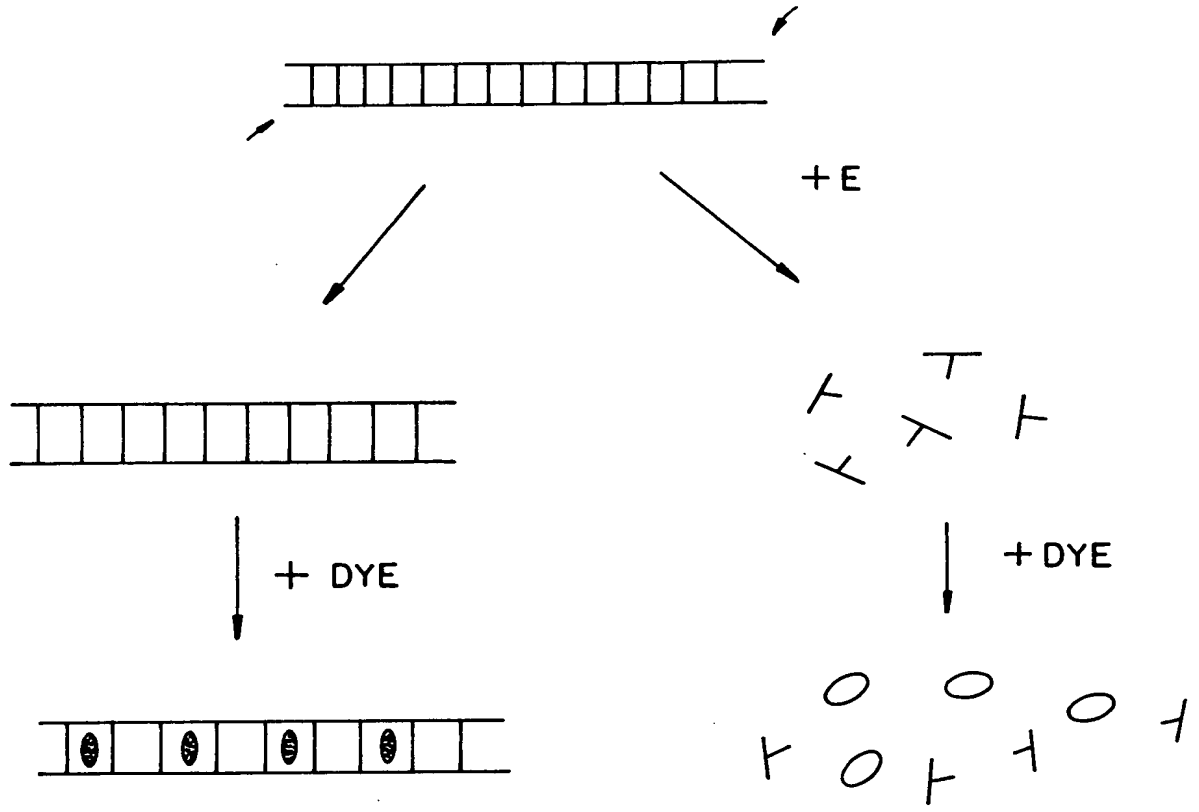
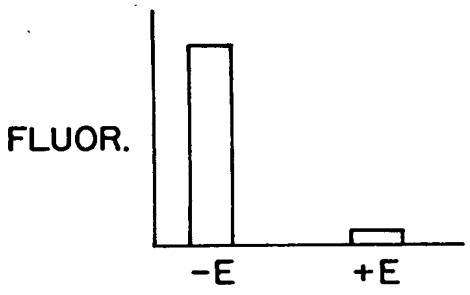


FIG. 31

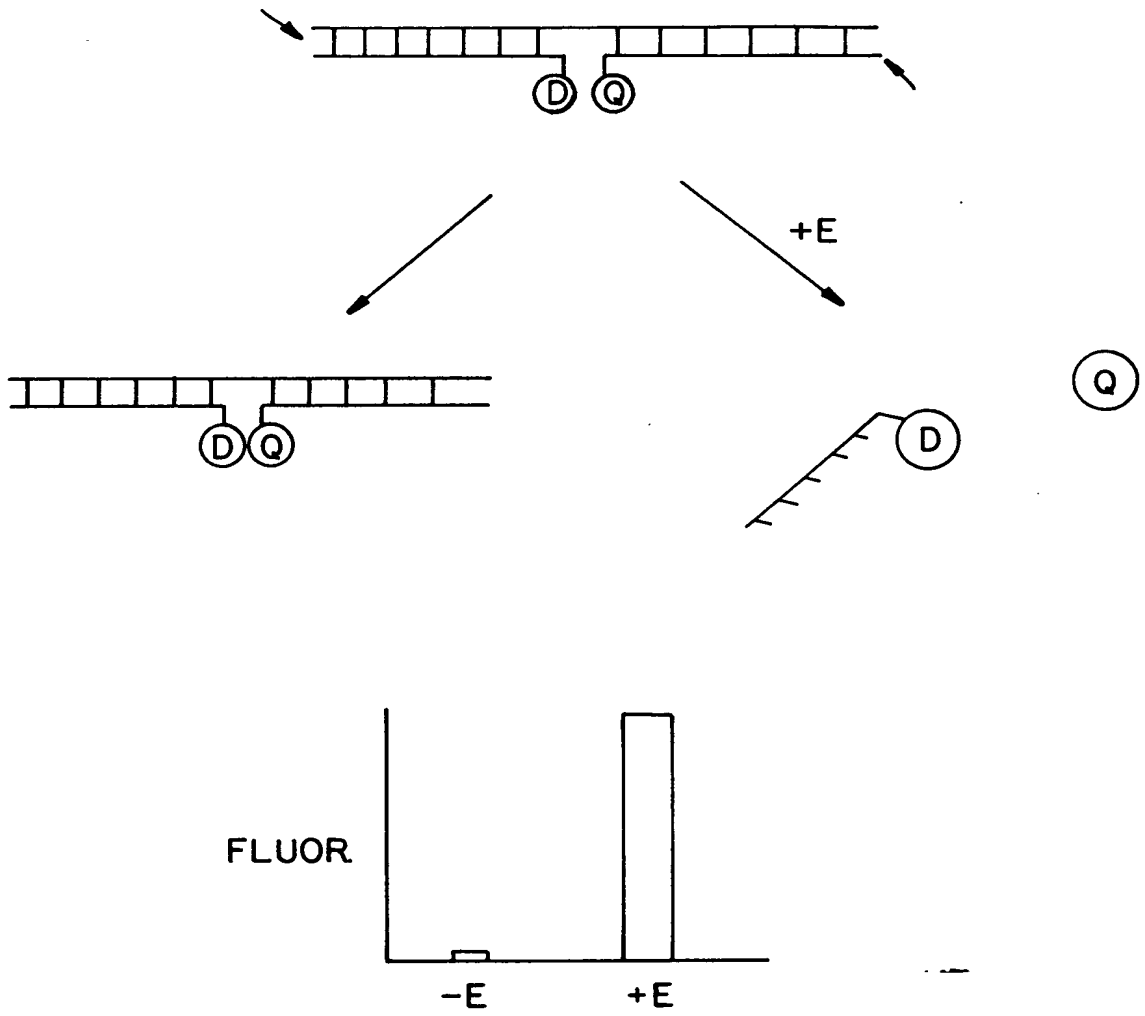
FIG. 32.





PICOGREEN DYE ASSAY

FIG. 33.



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